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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/593,914	06/14/2000	Jens J. Hyldig-Nielsen	BP9901US	8319

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EXAMINER
MYERS, CARLA J

ART UNIT	PAPER NUMBER
1634	16

DATE MAILED: 02/25/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/593,914	HYLDIG-NIELSEN ET AL.	
	Examiner	Art Unit	
	Carla Myers	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 December 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8,10-12,16,18,19,21-26,29,32,34,46-49,61,62 and 80-87* is/are pending in the application.

4a) Of the above claim(s) *34* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8,10-12,16,18,19,21-26,29,32,46-49,61,62 and 80-87* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

1. This action is in response to Paper No. 15, filed December 6, 2002. Applicants arguments presented in the response of Paper No. 15 have been fully considered but are not persuasive to overcome all grounds of rejection. All rejections not reiterated herein are hereby withdrawn. This action is made final.

2. This application contains claims of groups II-XI (SEQ ID NO: 2-11), as set forth in the Office action of Paper No. 9, drawn to an invention nonelected with traverse in Paper No. 12. A complete reply to the final rejection must include cancellation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-8 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998).

Kosse discloses dot blot and *in situ* hybridization methods for the detection and enumeration of *Dekkera bruxellensis*. Kosse teaches that the *in situ* hybridization method is performed using fluorescent labeled probes (e.g., TRITC or FLUOS) and that the dot blot hybridization is performed using digoxigenin labeled probes (page 469). The probes are complementary to sequences of the *Dekkera bruxellensis* 18S rRNA region (page 469; Table 2). The reference teaches that prior to *in situ* hybridization, yeast cell walls must be permeablized and that probes must be selected to yeast 18S rRNA regions which are fully accessible to probes (see page 478). Kosse teaches that *Dekkera bruxellensis* was successfully detected by in situ hybridization using 20% formaldehyde (see Table 2 and page 474). Table 2 lists additional 18S rRNA probes for the detection of other yeasts known to cause spoilage of dairy products (see page 468 and Table 2). Probes are also disclosed which are specific for all yeasts and for all eukaryotes (Table 2). Kosse further teaches that it is essential to provide accurate methods for detecting the presence of yeast in dairy products and other foods so as to ensure high quality and safe food products (see page 468).

Kosse teaches that the *in situ* hybridization method is performed using fluorescent- labeled probes and that dot blot hybridization is performed using digoxigenin labeled probes. Kosse does not specifically teach using enzyme-linked probes, or specifically soy bean peroxidase labeled probes.

However, Stender (1998) teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic

acid hybridization complexes (page 20). Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of Kosse so as to have specifically labeled the probes with soy bean peroxidase in order to have achieved the benefit of providing an effective means for labeling the probes, thereby facilitating the detection of *Dekkera bruxellensis.*

Response to arguments:

In the response of Paper No. 15, Applicants state that claim 1 and its dependent claims are limited to enzyme-linked in situ hybridization probes and not probes suitable for hybridization as the "Examiner appears to suggest at page 8 of the Office action." The examiner agrees that in view of the amendment to the claims set forth in Paper No. 15, the claims rejected above are limited to enzyme-linked in situ hybridization probes. Applicants state that the rejection is not proper and that no motivation or reasonable expectation of success has been provided for applying the enzyme-linked in situ probe to the detection of yeasts. However, as stated in the office action of Paper No. 13, there are no teachings in Amann which indicate that the enzyme linked probes cannot be applied to the detection of yeasts. Amann provides the results obtained when applying the enzyme-linked probes to the detection of S. cerevisiae. However, Amann does not teach that these results apply to the detection of all yeasts. While Amann teaches that an enzyme-labeled probe was not useful for the detection of Saccharomyces cerevisiae, the reference teaches that modifying the conditions for permeabilization of cells allows one to use enzyme-labeled probes for some organisms. The specification provides no teachings as to critical steps

that must be performed in order to allow for the detection of yeasts by in-situ hybridization using enzyme-labeled probes and the claims clearly do not recite any critical steps which distinguish the claims over prior art *in-situ* hybridization methods of detecting microorganisms using enzyme-labeled probes. Accordingly, given the teachings in the art of methods for detecting microorganisms using enzyme-labeled probes and the knowledge in the art of how to modify the conditions of permeabilization in order to allow for larger molecules to penetrate cells, the ordinary artisan would have had a reasonable expectation of success of applying the *in-situ* hybridization methods of Stender to the detection of yeast using enzyme labeled probes. Applicants response does not address why these teachings in the art would not have lead the ordinary artisan to the claimed invention and do not address why these combined teachings would not have provided a reasonable expectation of success.

4. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosse in view of Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, Kosse and Stender do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

In the response of Paper No. 15, Applicants traverse this rejection for the same reasons stated in paragraph 3 above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

5. Claims 1-8, 10-12, 16, 18-19, 21-26, 29, 32, 46, 61-62, 86 and 87 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and further in view of Stender et al (1998; reference BB).

De Wachter teaches an isolated nucleic acid consisting of the sequence of the 18S rRNA of *Dekkera/Brettanomyces bruxellensis*. The 18S rRNA of De Wachter comprises the sequence of SEQ ID NO: 1 (see nucleotides 1066-1052 of GenBank Accession No. X58052). The nucleic acid of De Wachter is considered to have the property of being suitable as a probe for the detection, identification or quantitation of *Dekkera/Brettanomyces bruxellensis*. De Wachter does not teach labeling the 18S rRNA with a detectable moiety, and particularly does not teach labeling the probe with an enzyme.

Kosse teaches hybridization methods, including dot blot hybridization and in situ hybridization, for the detection of *Dekkera bruxellensis*. Kosse teaches labeling probes with either chemiluminescent labels (e.g., digoxigenin) or fluorescent labels (e.g., TRITC or FLUOS) to facilitate the detection of yeasts and to particularly facilitate the detection of *Dekkera bruxellensis* (see page 469). The reference further exemplifies an 18S rRNA probe specific for *Dekkera bruxellensis* (see Table 2) and probes specific for other yoghurt spoiling yeasts. Kosse teaches

that it is essential to provide accurate methods for detecting the presence of yeast in dairy products, such as yoghurt, so as to ensure high quality and safe food products (see page 468).

In view of the teachings of Kosse, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have used the labeled 18S rRNA of De Wachter as a probe under suitable hybridization conditions in order to have facilitated the detection of *Dekkera bruxellensis* in dairy samples. Additionally, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have generated probe sets comprising one or more probes for *Dekkera bruxellensis* or comprising probes for *Dekkera bruxellensis* and probes for other yeast and to have labeled each probe with a different detectable moiety in order to have allowed for the detection and differentiation of multiple types of yeast in dairy products, such as yoghurt.

The combined teachings of De Wachter and Kosse do not teach labeling the probes with soy bean peroxidase. However, Stender teaches that enzyme labels, including soy bean peroxidase, may be used in place of fluorescent or chemiluminescent moieties to facilitate the detection of nucleic acid hybridization complexes (page 20). It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter in view of Kosse by using soy bean peroxidase to label the probes in order to have provided an equally effective probe for the detection of *Dekkera bruxellensis.*

Secondly, De Wachter and Kosse do not teach immobilizing the *D. bruxellensis* and/or yeast probes onto a solid support. However, Stender teaches that hybridization probes may be

immobilized onto solid supports and particularly may be in the format of an array (page 31). It is stated that the use of an array provides the advantage of allowing for the simultaneous analysis using 100 or more different probes. Accordingly, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have immobilized the probes of De Wachter and Kosse onto a solid support, as taught by Stender, in order to have achieved the benefit of simultaneously assaying for the presence of target sequences complementary to a multitude of different probes.

Thirdly, the combined references do not teach PNA probes for the detection of *Dekkera bruxellensis*. However, Stender (see, for example, pages 3 and 10-11) teaches PNA probes complementary to rRNA sequences which are useful for the detection of microorganisms. Stender teaches that PNA probes hybridize to RNA or DNA with a higher affinity and specificity than their nucleic acid counterparts. PNA probes are also more stable due to their resistance to naturally occurring nucleases and proteases. Methods are disclosed for modifying nucleic acid probes so as to incorporate peptide nucleic acid moieties (see, for example, pages 13-14). Stender also teaches that PNA probes can be used in either *in situ* or *in vitro* hybridization methods (page 23). In view of the teachings of Stender, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probes of De Wachter and Kosse by including peptide nucleic acid moieties in the probes and thereby generating PNA probes, in order to have provided probes with increased affinity and specificity and increased resistance to nucleases and proteases.

With respect to claim 25, De Wachter and Kosse do not teach adding blocking probes to the probe sets. Stender (page 25, 28) teaches adding blocking probes (i.e., random non-selected probes) to hybridization reactions in order to reduce non-specific binding. It would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the probe sets of De Wachter and Kosse so as to have included the "blocking probes" disclosed by Stender in order to have accomplished the objective of reducing non-specific binding of the yeast probes.

Response to arguments:

In the response of Paper No. 15, Applicants traverse this rejection by stating that Kosse teaches that only the 3' end of the 18S rRNA is accessible to fluorescently labeled probes and that other variable regions are not accessible to in-situ hybridization. Applicants conclude that it is not reasonable to expect that any nucleotide which is homologous to the gene sequence described by De Wachter can be used to produce in situ hybridization probes.

Applicants arguments have been fully considered but are not persuasive. Firstly, it is noted that while claims 1-9 are limited to in situ hybridization probes and claim 46 is limited to a method of in situ hybridization, the remaining claims are not limited to in situ hybridization probes or to methods of in situ hybridization. Accordingly, Applicants are arguing limitations that are not recited in claims 10-12, 16, 18-19, 21-26, 29, 32, 61-62, 86 and 87. All of the probes disclosed by Kosse were found to be useful for dot blot hybridization, regardless of whether they were derived from the 5' end or 3' end of the rRNA. Secondly, Applicants claims recite the language of

"comprising" and thereby include the 5' sequences of the rRNA. There are no teachings in Applicants specification which indicate that the probes should consist of only the 3' end of the rRNA and no guidance provided in the specification which would indicate that the 5' end of the rRNA should be excluded from the hybridization probes. Thirdly, Kosse teaches that some regions of the 5' 18S rRNA may not be useful as species-specific probes because the target region may not be accessible for hybridization. However, Kosse does not provide any comments regarding probes which include both the 5' end and 3' end of the 18S rRNA. Thereby, Applicants have not established that the probe of De Wachter labeled with an enzyme moiety would not be suitable for in-situ hybridization or other types of hybridization.

6. Claims 47-49 and 80-85 are rejected under 35 U.S.C. 103(a) as being unpatentable over De Wachter in view of Kosse and Stender (1998) and further in view of Parton (U.S. Patent No. 5,905,038).

The teachings of De Wachter, Kosse and Stender are presented above. The combined references do no teach filtering the microorganism and culturing the microorganism on a filter prior to performing in situ hybridization.

Parton (col. 1-2) teaches methods for isolating and analyzing samples for the presence of microorganisms wherein the methods comprise filtering a sample containing microorganisms through a membrane filter so as to trap the microorganisms on the filter and then culturing the microorganisms on the filter.

In view of the teachings of Parton, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Kosse in view of Stender so as to have isolated the microorganisms by filtration and then cultured the microorganisms on the filter prior to performing in situ hybridization in order to have provided an effective means for purifying and concentrating the microorganisms and for increasing the number of microorganisms to allow for the formation of colony forming units so as to increase the accuracy and sensitivity of the detection method.

Furthermore, the combined references do not teach kits comprising filters and culture media. However, the method of Kosse in view of Stender requires the use of fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, and film. In view of the teachings of Parton, modification of the method of De Wachter, Kosse and Stender to include a filtration and culturing step would have resulted in a method which further required the use of a filter and culture media. Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have packaged the fixation solutions, soy bean peroxidase labeled PNA probes, wash solutions, enzyme substrates for soy bean peroxidase, film, filter and culture media in a kit for the expected benefits of convenience and cost-effectiveness for practioners in the art wishing to detect *Dekkera bruxellensis*.

Response to arguments:

In the response of Paper No. 15, Applicants traverse this rejection for the same reasons stated in paragraph 5 above. Accordingly, the response to those arguments applies equally to the present grounds of rejection.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carla Myers whose telephone number is (703) 308-2199. The examiner can normally be reached on Monday-Thursday from 6:30 AM-5:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, W. Gary Jones, can be reached on (703)-308-1152. The fax number for the Technology Center is (703)-305-3014 or (703)-305-4242.

Any inquiry of a general nature or relating to the status of this application should be directed to the receptionist whose telephone number is (703) 308-0196.

Carla Myers

February 24, 2003

Carla Myers

CARLA J. MYERS
PRIMARY EXAMINER